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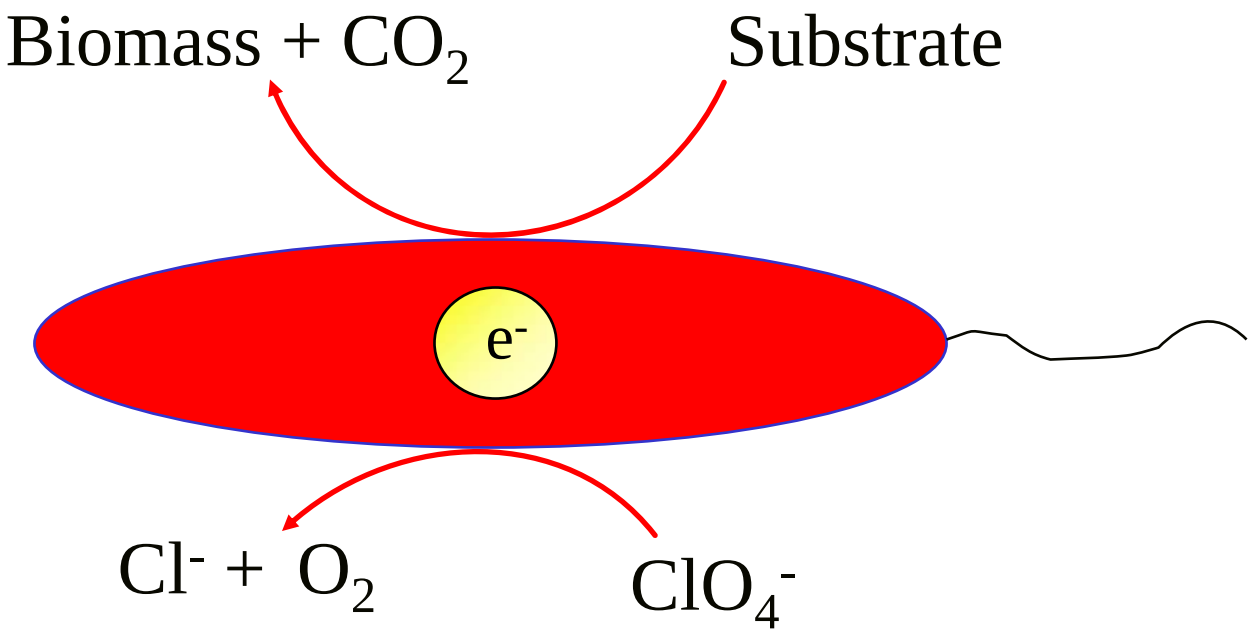
BIOTREATMENT OF PERCHLORATE IN GROUNDWATER

Paul B. Hatzinger, Ph.D.
Envirogen, Inc.

November 29, 2000

Biological Perchlorate Reduction



BIOLOGICAL PERCHLORATE REDUCTION

- ***Terminal Electron Acceptor:***

$$\underset{\text{(perchlorate)}}{ClO_4^-} \longrightarrow \underset{\text{(chlorate)}}{ClO_3^-} \longrightarrow \underset{\text{(chlorite)}}{ClO_2^-} \longrightarrow O_2 + Cl^- \searrow H_2O$$

- ***Highly Favorable Reaction (kJ/mol acetate)***

  $-\Delta G = 844$ $(O_2 \longrightarrow H_2O)$

  $-\Delta G = 801$ $(ClO_4^- \longrightarrow ClO_2^-)$

  $-\Delta G = 792$ $(NO_3^- \longrightarrow N_2)$

- ***Several Microbial Isolates Reported***

  Strain CBK (Bruce et al., 1999); Strain perace1 (Herman et al., 1999)
  Strain GR-1 (Rikken et al., 1996)
  Wolinella succinogenes HAP-1 (Wallace et al., 1996)
  Ideonella dechloratans (Malvquist et al., 1994)
  Vibrio dechloraticans (Korenkov et al., 1976)

Biological Treatment

- ***Ex Situ* Treatment**

  Fluidized Bed Reactor - Full Scale - (Aerojet/Envirogen).

  Suspended Growth Reactor - Full Scale - (USAF/ARA).

  Fixed Film Reactor- Pilot Scale - (USAF/Wallace et al, 1998).

- ***In Situ* Treatment**

  Laboratory and Pilot Studies Show Promise for *In Situ* Biostimulation (Envirogen/Geosyntec).

  One Full-Scale System - McGregor NWIRP.

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EX SITU TREATMENT

FLUIDIZED BED REACTOR

AEROJET/ENVIROGEN

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FBR Flow Schematic

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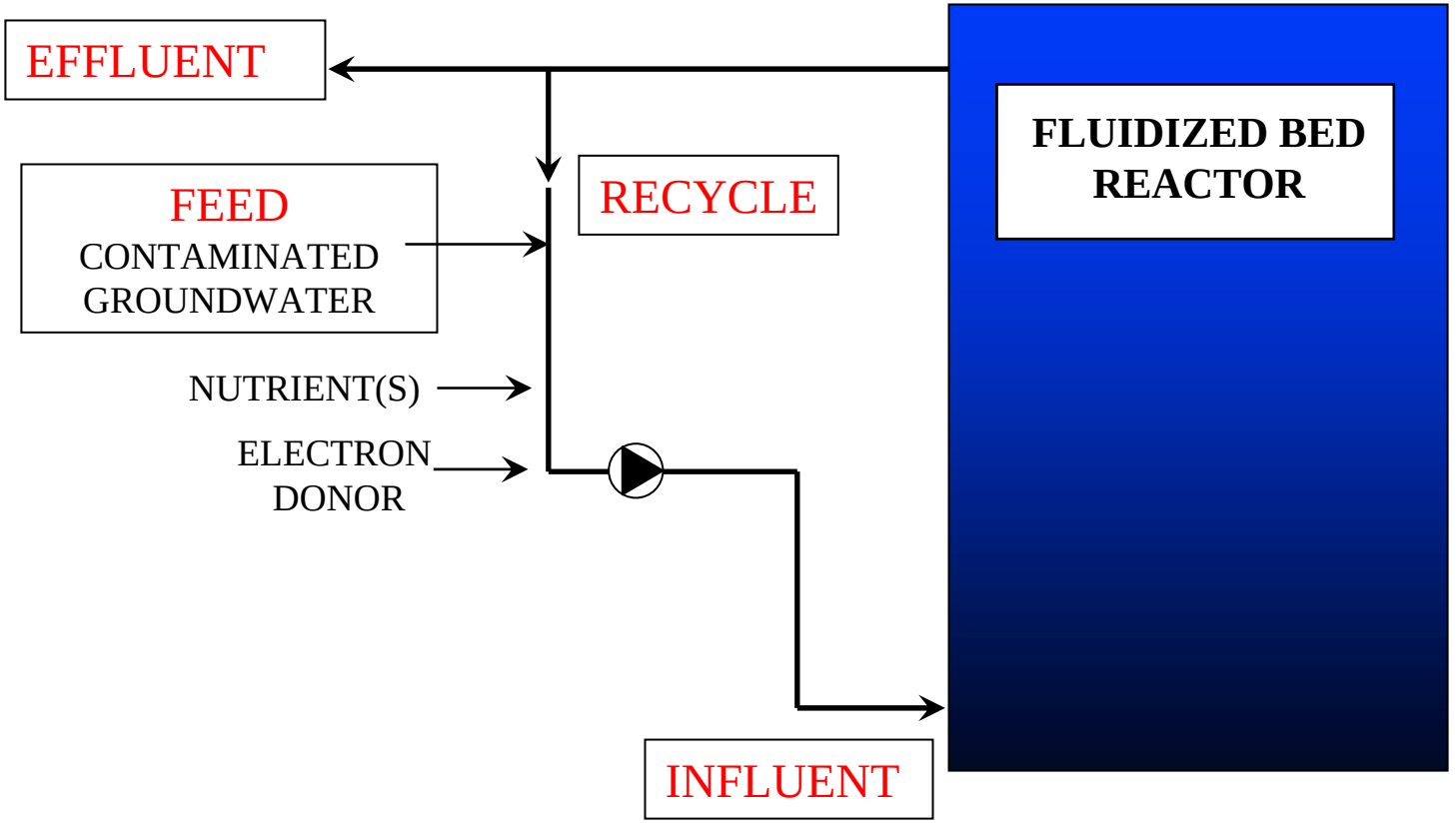

Aerojet Facility - Rancho Cordova California

- FBR SYSTEM
  - 4 - 6 mg/L perchlorate
  - 4,000 GPM flow rate
  - Four 14 ft diameter units
  - Ethanol as electron donor
  - GAC media


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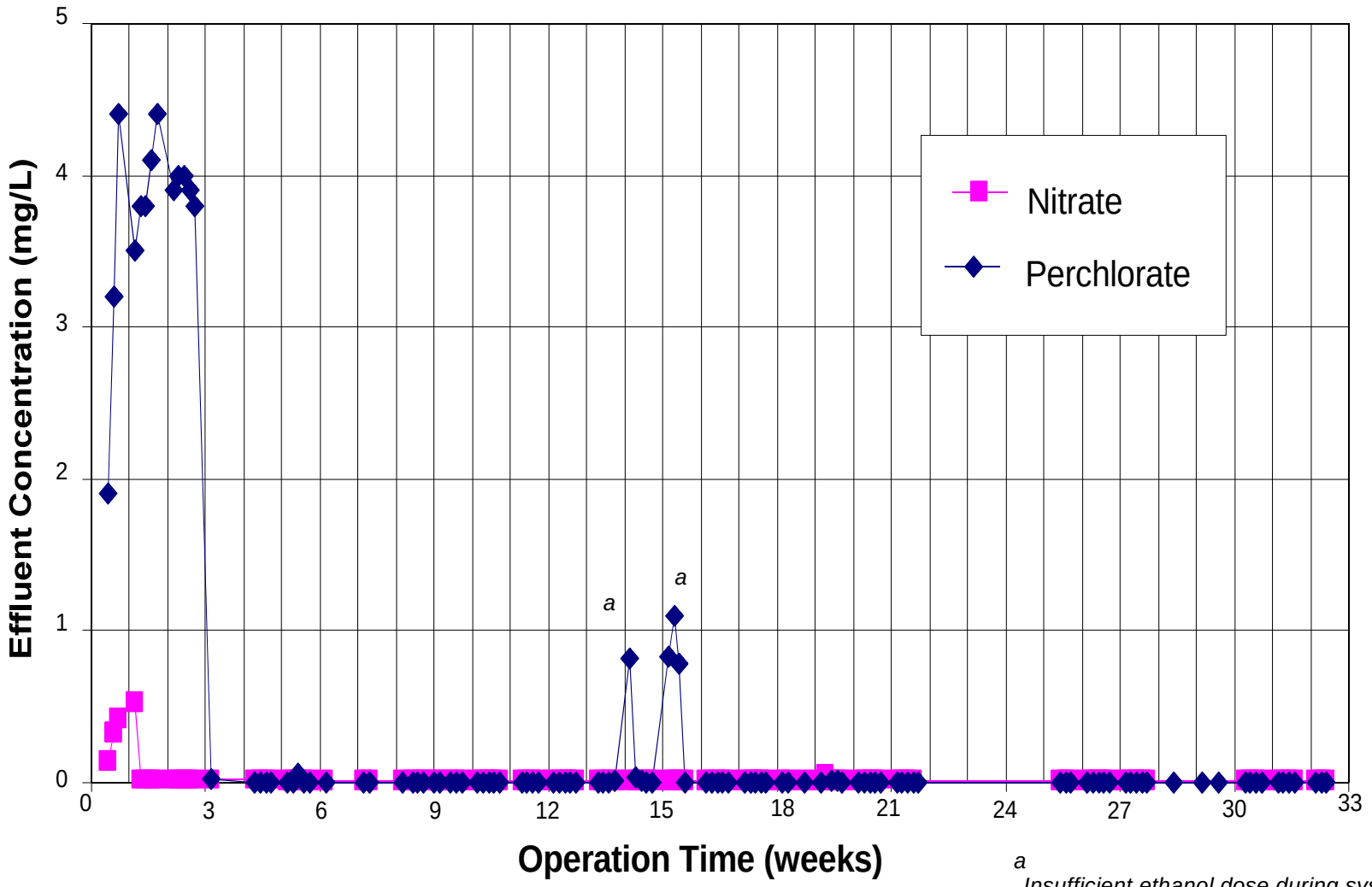
Full-Scale FBR Performance



a *Insufficient ethanol dose during system tests.*

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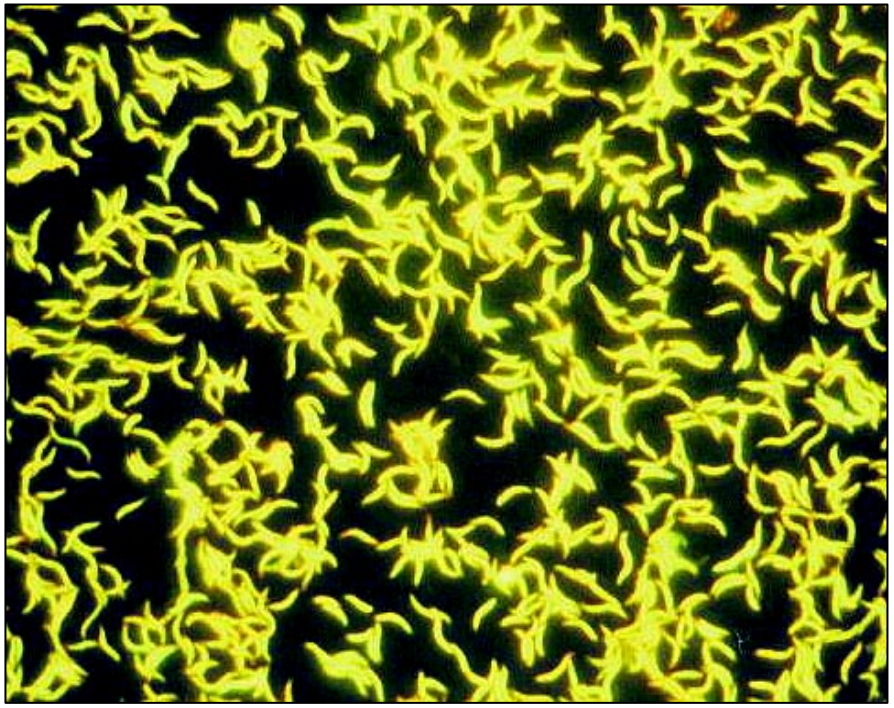
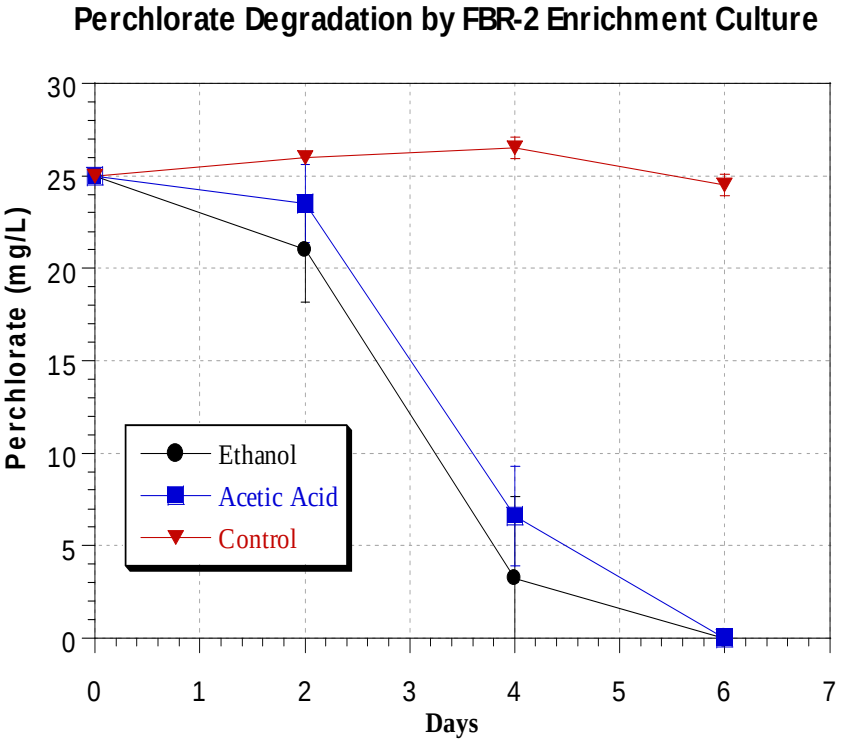
Pure Culture

Dechlorospirillum sp. FBR2





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IN SITU TREATMENT

SERDP PROJECT CU-1163

(March 2000 - December 2001)

In Situ Perchlorate Bioremediation

Key Question for Technology Development: **Why does perchlorate persist in groundwater?**

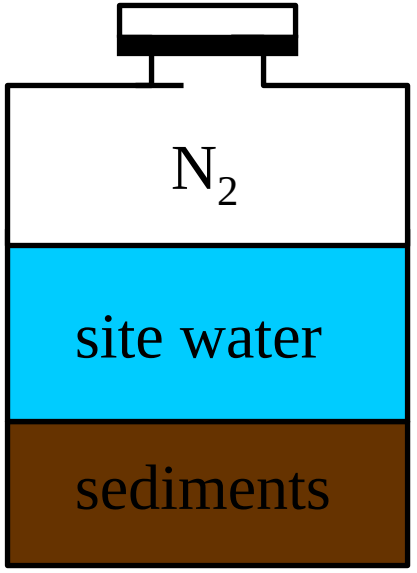
Hypotheses:

- **Absence of suitable electron donor (substrate)?**
- **Inhibition by alternate electron acceptors?**
- **Lack of indigenous bacteria capable of perchlorate reduction?**
- **Unfavorable environmental conditions?**

Aquifer Microcosms

Serum Bottles:

- Site Sediments
- Site Groundwater



Tests:

1. Electron Donors
2. Alternate Electron Acceptors
3. Environmental Variables

RESEARCH SITES

(1) JET PROPULSION LAB (CA).*

(2) INDIAN HEAD NSWC (MD) *(2 Sites)*.*

(3) ROCKY MT COMMERCIAL SITE.*

(4) OYSTER VIRGINIA (*Pristine Site*).*

(5) LONGHORN AAP (TX).

**** Studies Underway or Complete***

Rocky Mountain Site Groundwater Characteristics

1. **Depth 89 - 99 ft (BLS)**
2. **Geochemistry**

Perchlorate	**60 mg/L**
Nitrate	**23 mg/L**
Sulfate	**364 mg/L**
Chloride	**2,500 mg/L**
TDS	**5,000 mg/L**
pH	**7.3**
Co-Contaminants	**TCE, TCA, DCE, Cr**

Perchlorate Degradation in Groundwater Microcosms from the Rocky Mountain Site

Treatment	Perchlorate Concentration (mg/L)[1]				
Electron Donors	***Day 0***	***Day 6***	***Day 14***	***Day 22***	***Day 35***
Killed	57 ± 2	60 ± 2	53 ± 2	60[3]	55[3]
No Addition	57 ± 2	60 ± 1	53 ± 1	53 ± 2	54 ± 1
Nitrogen/Phosphorus only	57 ± 2	62 ± 5	55 ± 1	59 ± 1	55 ± 2
Hydrogen	57 ± 2	61 ± 1	63 ± 10	52 ± 1	54 ± 1
Propane	57 ± 2	62 ± 0	66 ± 1	49 ± 0	53 ± 0
Benzoate	57 ± 2	62 ± 2	62 ± 1	49 ± 3	48 ± 2
Ethanol	57 ± 2	59 ± 3	63 ± 2	51 ± 0	43 ± 1
Methanol	57 ± 2	62 ± 2	63 ± 1	46 ± 4	47 ± 6
Acetate (no N or P)	57 ± 2	60 ± 5	54 ± 0	59 ± 1	49 ± 1
Acetate	**57 ± 2**	**62 ± 1**	**31 ± 6**	**2 ± 2**	**< 0.5**
Yeast Extract/Ethanol	**57 ± 2**	**60 ± 0**	**1 ± 1**	**< 0.5**	**NS[2]**
Lactate	**57 ± 2**	**60 ± 1**	**< 0.5**	**< 0.5**	**NS**
Molasses	**57 ± 2**	**59 ± 1**	**< 0.5**	**< 0.5**	**NS**
Sucrose	**57 ± 2**	**61 ± 1**	**< 0.5**	**< 0.5**	**NS**
Inoculum Added					
Culture FBR2 + Etoh	**57 ± 2**	**15 ± 1**	**< 0.5**	**< 0.5**	**NS**

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IHDIV Naval Surface Warfare Center

Building 1170

300-Gallon Mixer

Washdown Water Discharge

1998 - Offsite Disposal

IHDIV Hogout Facility

1982 - 1994 (discharge)

1996 - Present (recycle)

IHDIV Naval Surface Warfare Center

Sample Characteristics

	Hogout	Building 1170
1. Depth	6 - 13 ft (BLS)	4 - 12 ft (BLS)
2. Perchlorate	25 mg/L (water)	< 0.004 mg/L (water)
	45 mg/L (slurry)	< 0.004 mg/L (slurry)
3. pH	4.8/4.3 (w/s)	5.9/6.1 (w/s)
4. Alkalinity	19	40 mg/L
5. Sulfate	88 mg/L	12 mg/L
6. Nitrate	< 0.4 mg/L	< 0.2 mg/L
7. Nitrite	< 0.4 mg/L	< 0.2 mg/L
8. Chloride	26 mg/L	43 mg/L
9. Co-Contaminants	NA (binder/metals?)	fuel ?

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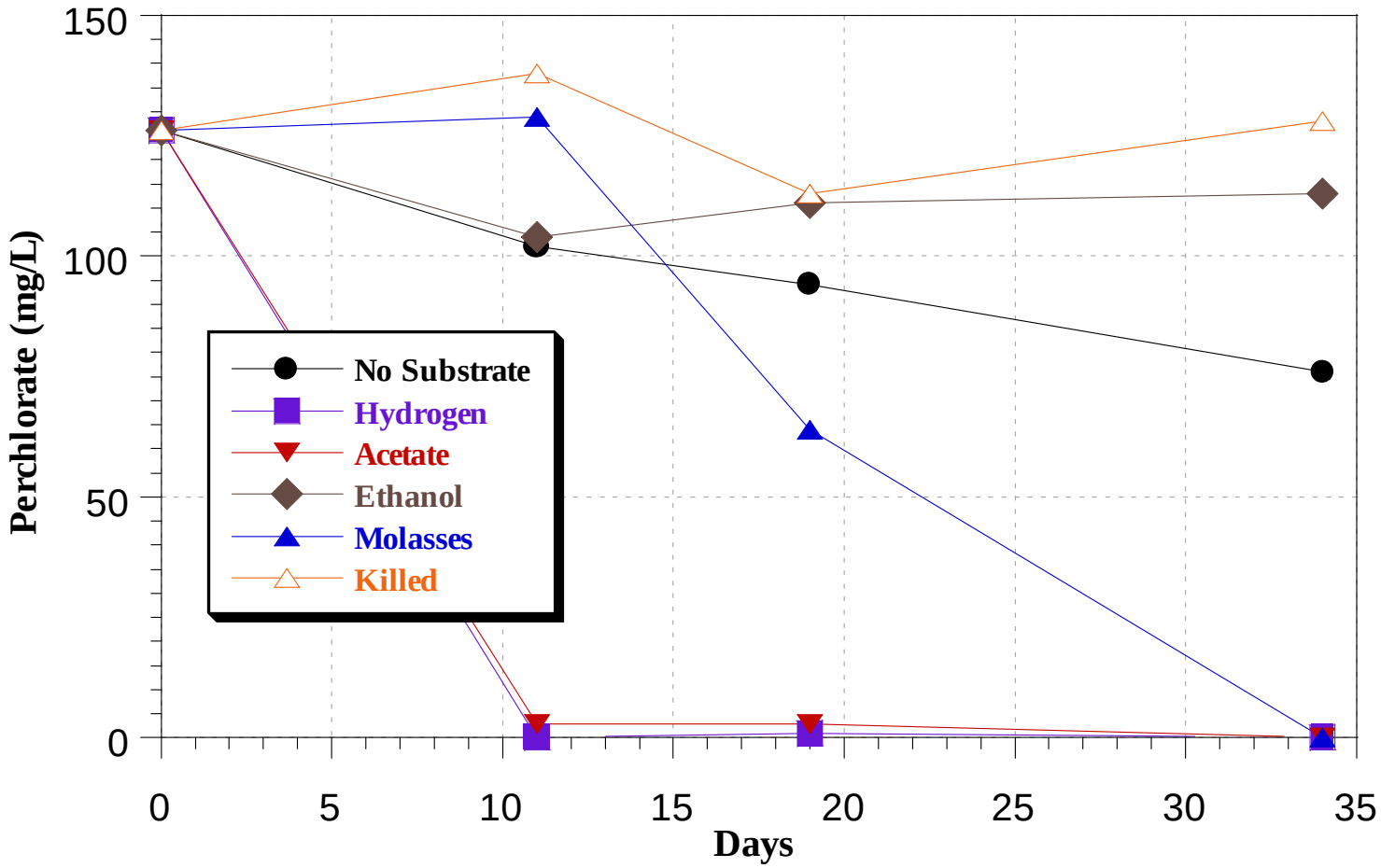
Influence of Electron Donors on Perchlorate Degradation in Aquifer Microcosms from IHDIV Building 1170 Site.



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Perchlorate Degradation in Sediment/Groundwater Microcosms from the IHDIV Hogout Site

Treatment	Perchlorate Concentration (mg/L)				
Electron Donors	***Day 0***	***Day 11***	***Day 20***	***Day 36***	***Day 71***
Killed Control	42 ± 4	41 ± 1	44 ± 2	36 ± 4	37 ± 2
No Substrate	42 ± 4	37 ± 1	36 ± 4	38 ± 1	39 ± 5
Nutrients Only	42 ± 4	38 ± 2	41 ± 4	42 ± 1	34 ± 1
Hydrogen	42 ± 4	38 ± 2	40 ± 4	32 ± 5	35 ± 2
Propane	42 ± 4	38 ± 1	39 ± 2	34 ± 2	37 ± 2
Ethanol	42 ± 4	39 ± 2	41 ± 2	36 ± 4	36 ± 3
Methanol	42 ± 4	41 ± 2	41 ± 1	32 ± 2	34 ± 2
Acetate	42 ± 4	39 ± 1	42 ± 2	33 ± 1	37 ± 1
Benzoate	42 ± 4	40 ± 1	43 ± 0	32 ± 1	38 ± 1
Lactate	42 ± 4	38 ± 3	43 ± 3	33 ± 2	37 ± 2
Molasses	42 ± 4	43 ± 2	43 ± 2	28 ± 1	36 ± 2
Sucrose	42 ± 4	44 ± 1	45 ± 0	31 ± 0	35 ± 0
Yeast Extract/Ethanol	42 ± 4	43 ± 2	44 ± 2	35 ± 3	37 ± 2
Bioaugmentation					
Inoculum FBR2[2]+ Etoh	42 ± 4	41 ± 1	44 ± 3	36 ± 2	36 ± 2

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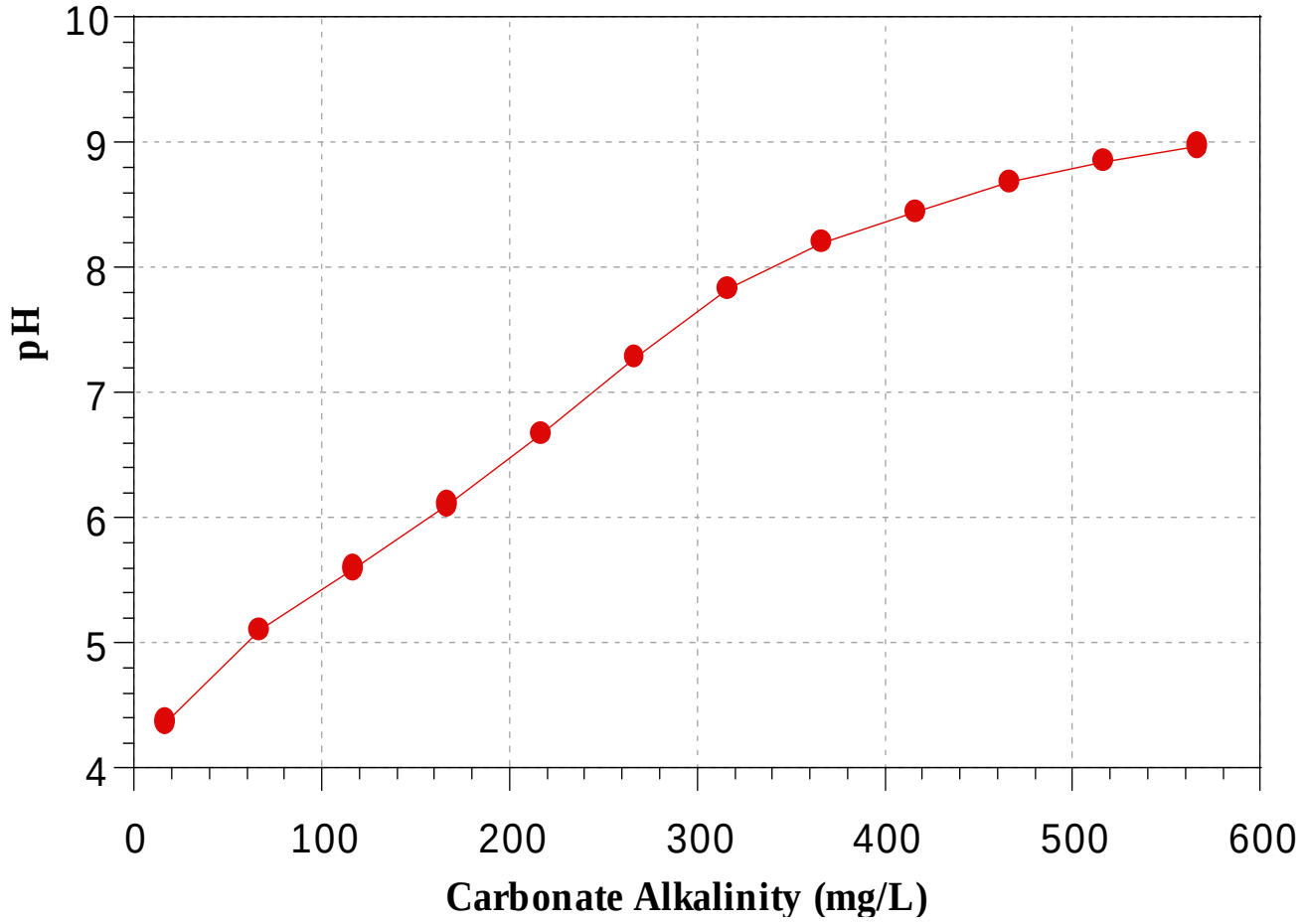
Carbonate Titration Curve for Sediment Slurries from IHDIV Hogout Site.



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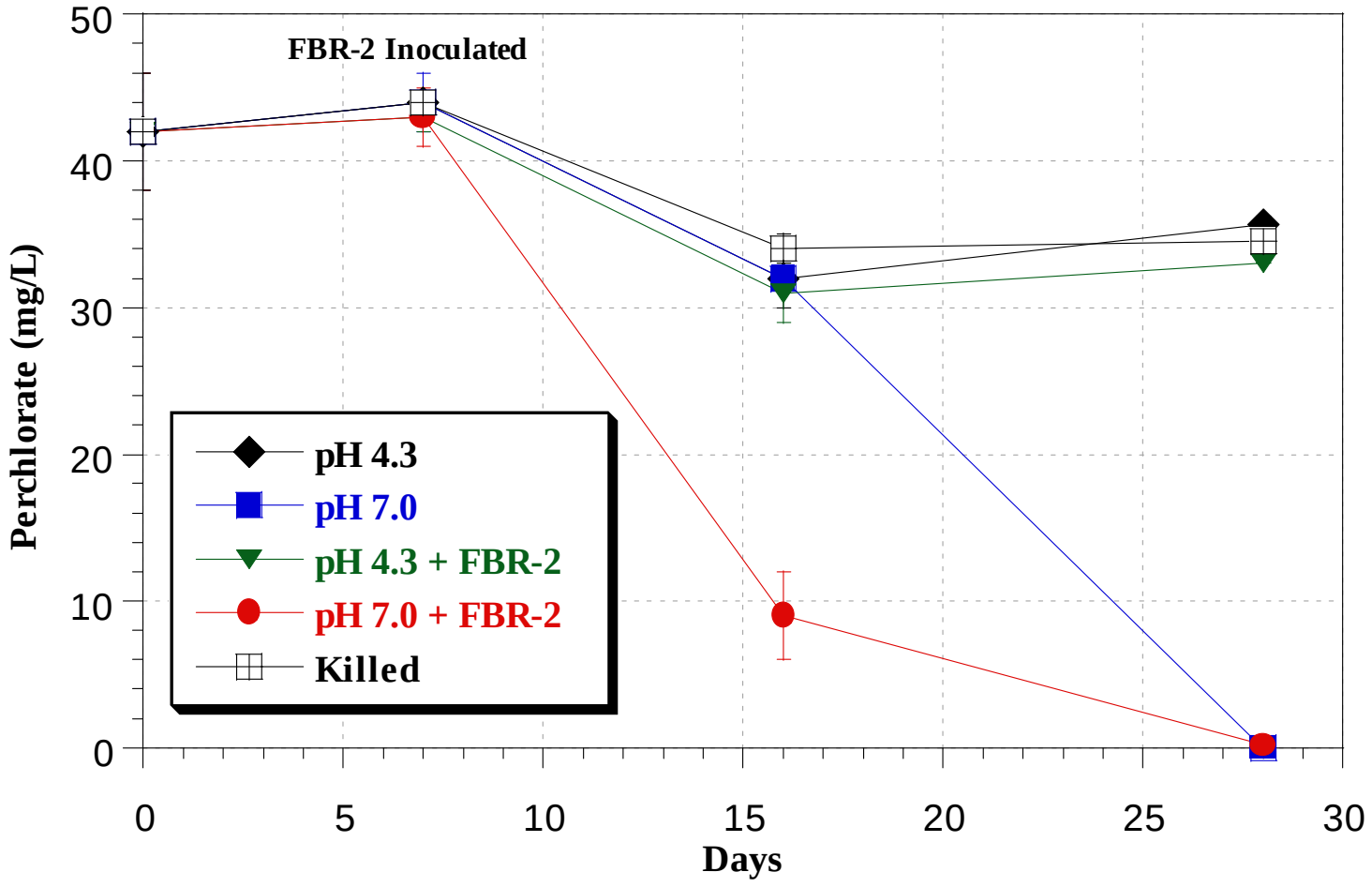
Influence of pH on Perchlorate Degradation in Aquifer Microcosms from the IHDIV Hogout Site



JPL Groundwater Characteristics (MW-7)

1. **Depth - 225 - 275 ft (BLS)**
2. **Geochemistry**

Perchlorate	**307 ug/L**
Nitrate	**18.6 mg/L**
Oxygen	**2.6 mg/L**
Sulfate	**44 mg/L**
Alkalinity	**140 mg/L**
pH	**7.6**
Other	**CT (50 ug/L), CF (5 ug/L)**
	TCE(15 ug/L), PCE (2 ug/L)

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Perchlorate Degradation in JPL Aquifer Microcosms Amended with Electron Donors or Perchlorate-Degrading Bacteria

Treatment	Perchlorate Concentration (µg/L)		
Electron Donors	***Day 0***	***Day 10***	***Day 21***
Killed Control	310 ± 0	293 ± 6	320 ± 0
Benzoate	310 ± 0	297 ± 6	150 ± 135
Methanol	310 ± 0	77 ± 57	< 5
Hydrogen	310 ± 0	177 ± 61	< 5
Propane	310 ± 0	283 ± 6	< 5
No Addition	310 ± 0	14 ± 19	< 5
Sucrose	310 ± 0	92 ± 67	< 5
Ethanol	310 ± 0	< 5	NS
Lactate	310 ± 0	< 5	NS
Molasses	310 ± 0	< 5	NS
Yeast Extract/Ethanol	310 ± 0	< 5	NS
Acetate	310 ± 0	< 5	NS
Bacteria Added			
Killed + Culture FBR2	310 ± 0	385 ± 7	415 ± 7
Culture FBR2+ YE/Etoh	310 ± 0	< 5	NS
Culture FBR2+ Acetate	310 ± 0	< 5	NS

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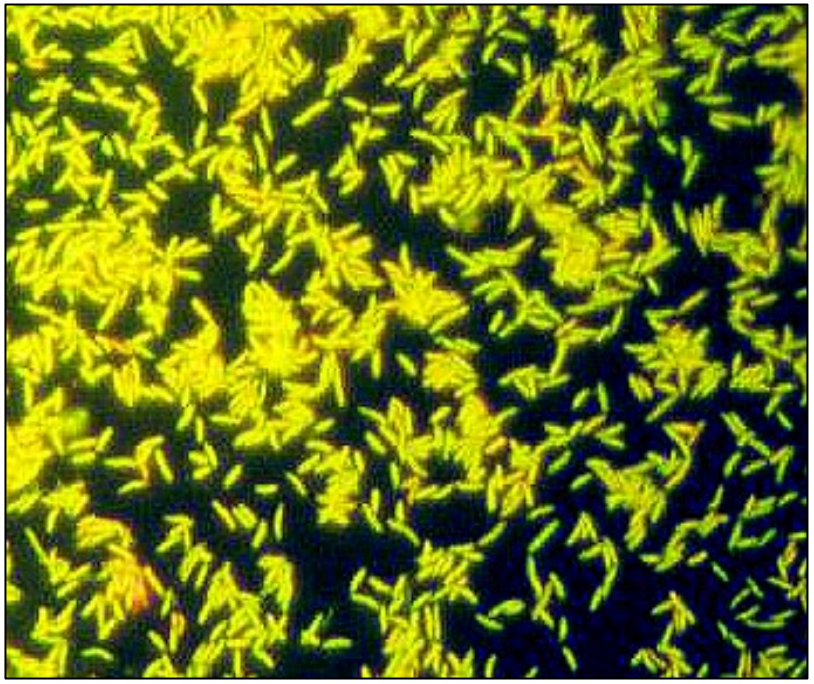

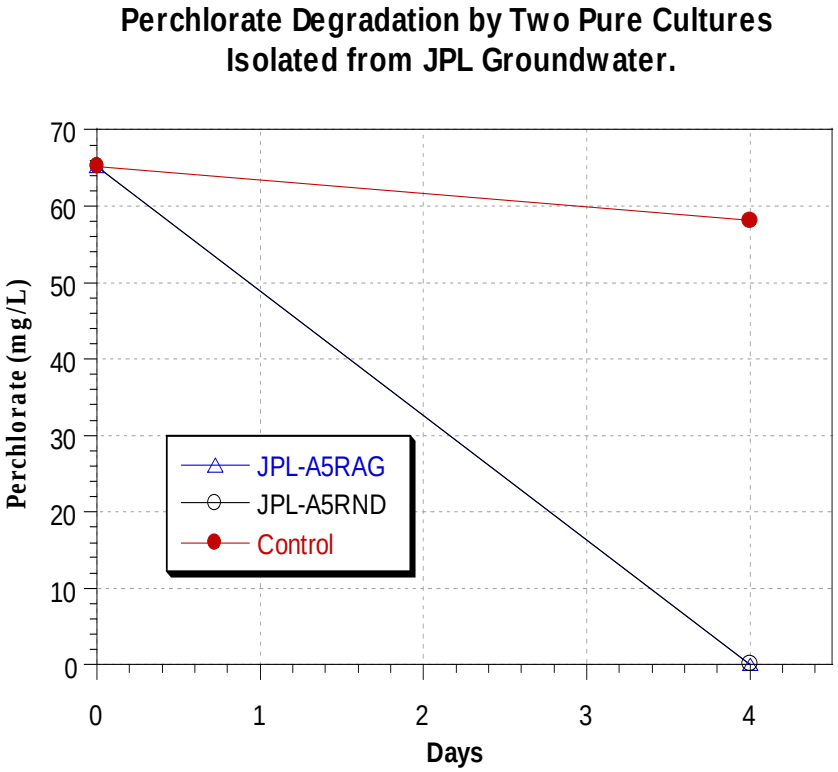

Dechlorisoma suilla JPL-A5RAG/JPL-A5RND

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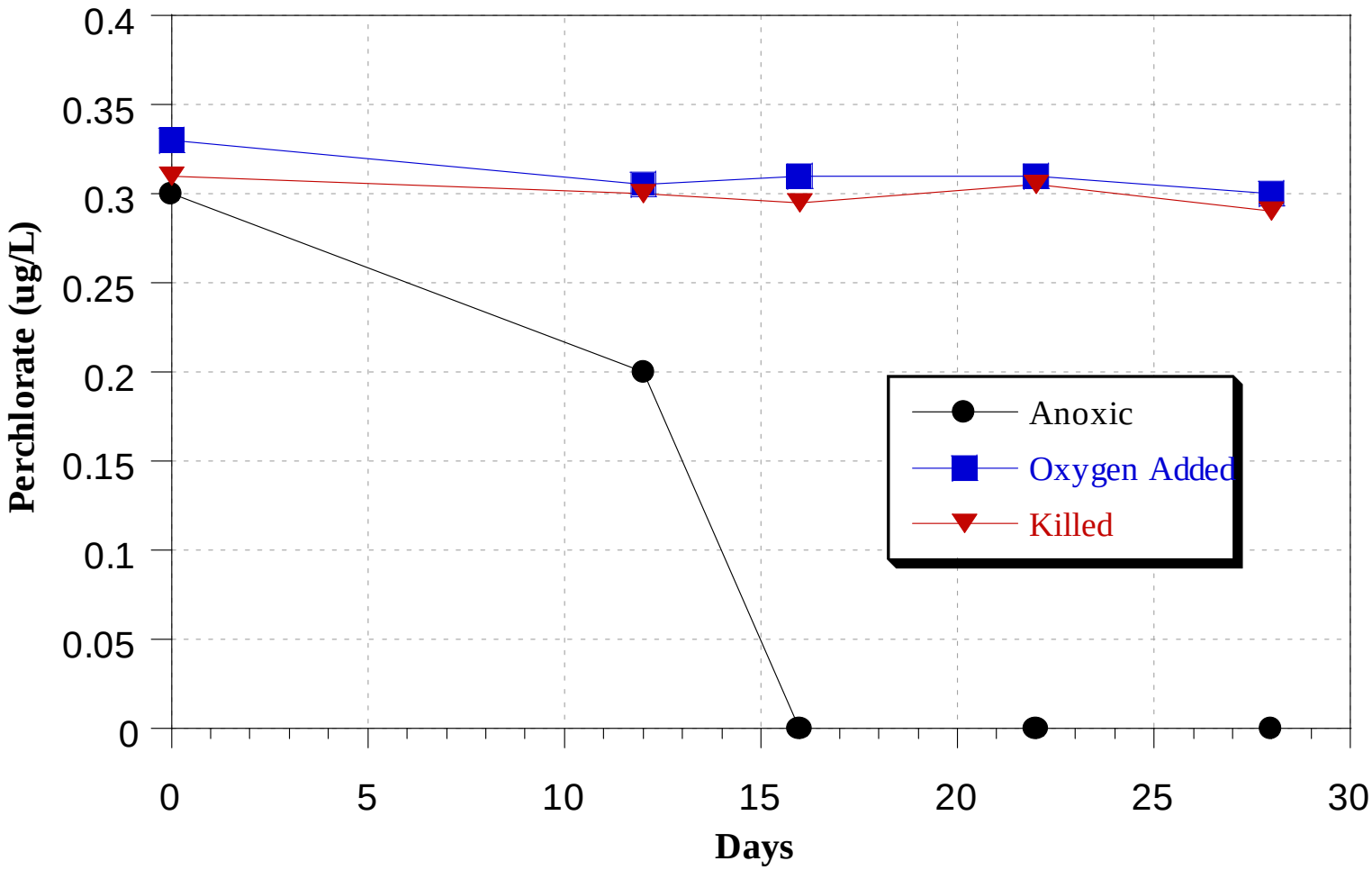
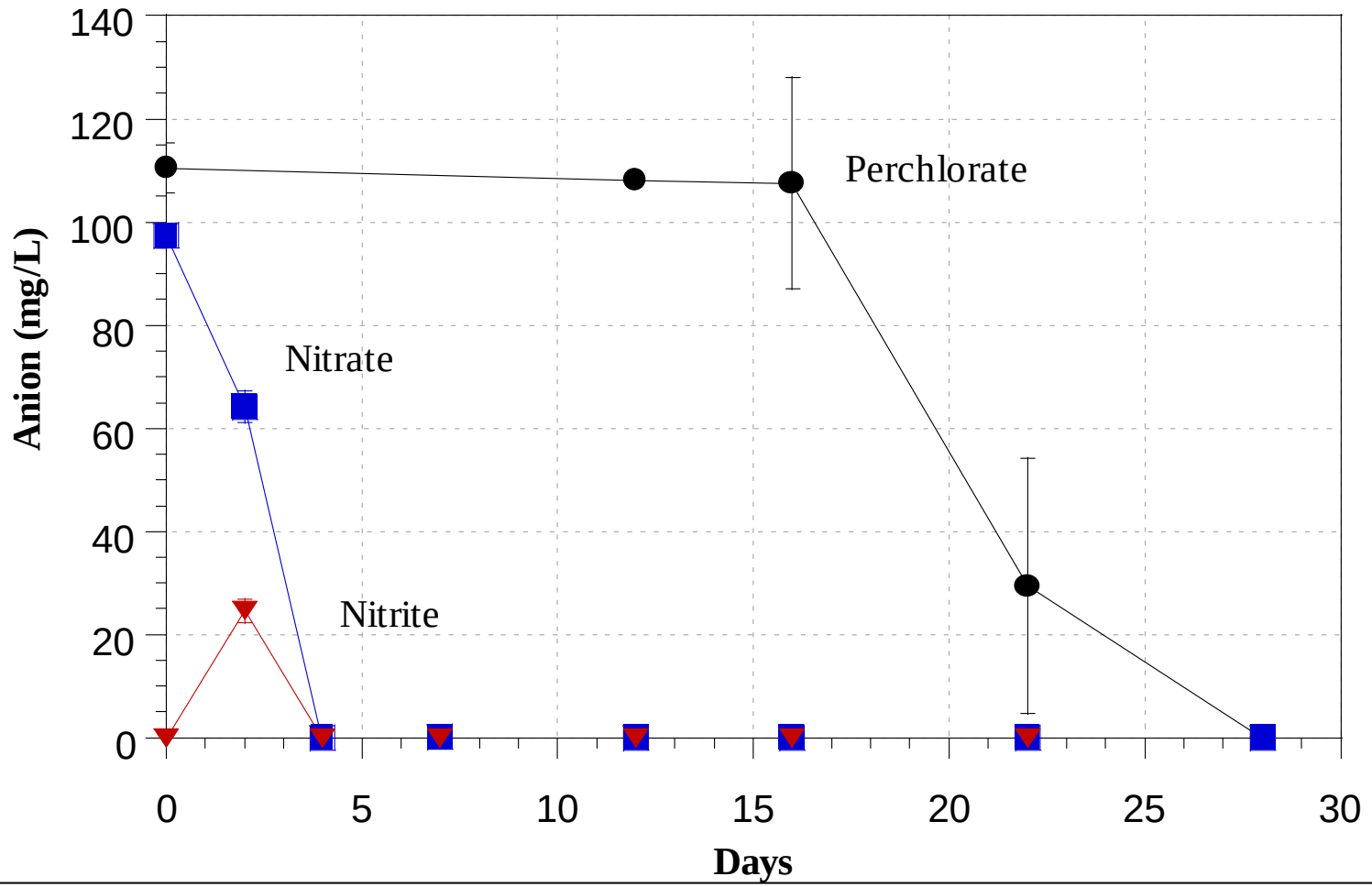
Influence of Oxygen on Perchlorate Degradation in Aquifer Microcosms from JPL



RESULTS

Degradation of Perchlorate (100 mg/L) and Nitrate (100 mg/L) in Aquifer Microcosms from JPL with Ethanol as a Substrate



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CONCLUSIONS

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Substrate	Jet Propulsion Lab	Rocky Mountain	Indian Head (Bldg 1170)	Indian Head (Hogout)
Hydrogen				
Propane			NA	
Acetate				
Lactate			NA	
Benzoate			NA	
Methanol			NA	
Ethanol				
Molasses				
YE/Ethanol			NA	
Sucrose			NA	
FBR2-Culture				

Rapid Biodegradation (≤ 14 Days)
Slow Biodegradation (≥ 14 Days)
No Biodegradation

Conclusions from Microcosm Studies

- **Electron Donor Addition Promising *In Situ* Approach**
- **Choice of Electron Donor Site Specific**
- **Low pH (< 5) Inhibitory to Perchlorate Degradation**
- **Oxygen Inhibitory to Perchlorate Degradation**
- **Nitrate and Nitrite Degraded before Perchlorate**

IN SITU TREATMENT OPTIONS

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In Situ Treatment Options

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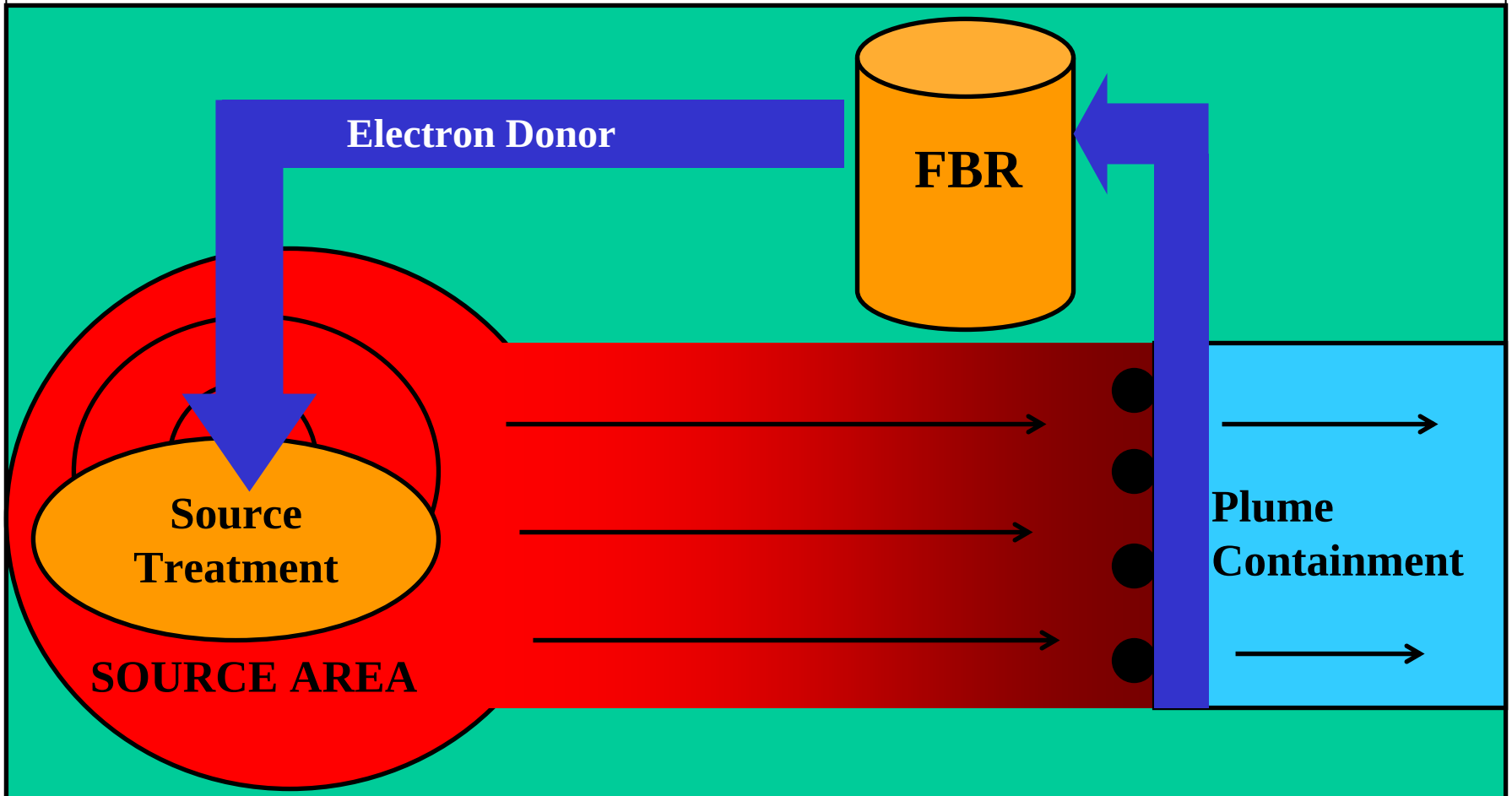

INTEGRATED IN SITU AND EX SITU TREATMENT SYSTEM

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In Situ Treatment

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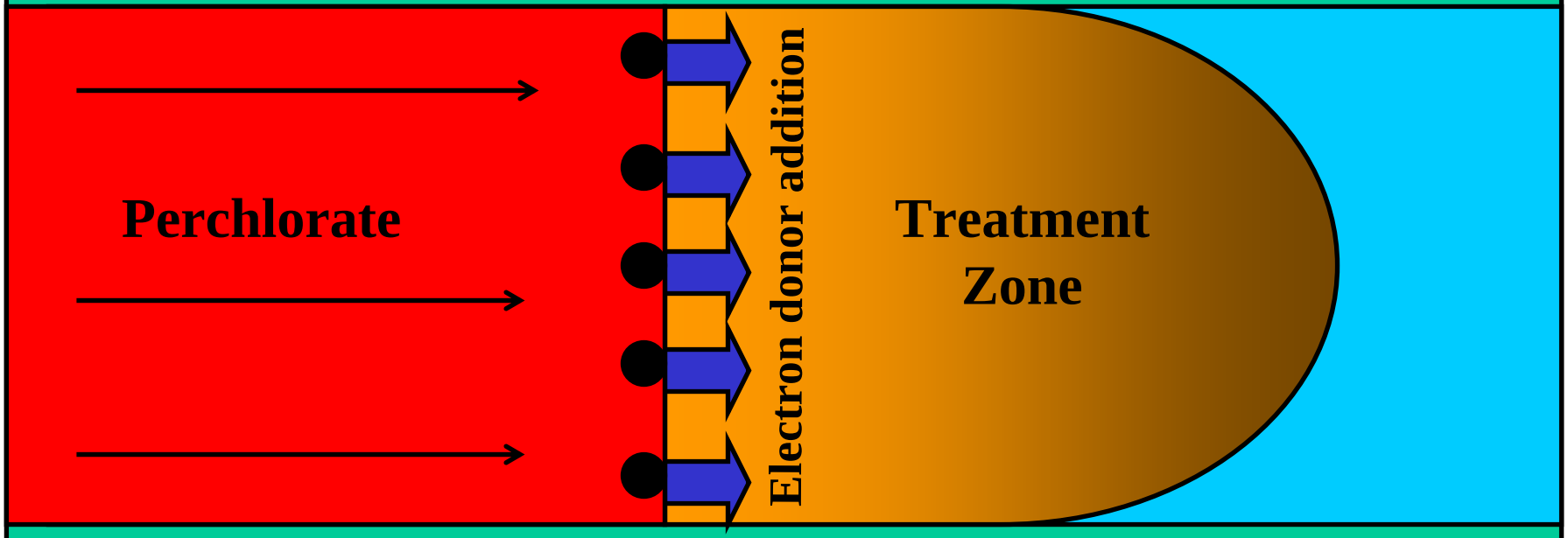
Passive Flow-Through Biobarrier



REACTIVE BARRIER TECHNOLOGY - FIELD SYSTEM

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In Situ vs *Ex Situ* Treatment Both !

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Factors

- Depth to Groundwater
- Plume Characteristics
- Aquifer Geochemistry
- Co-Contaminants
- Hydraulic Control
- Economics
- Waste Generation
- Water Use and Reuse
- Political Considerations
- Social Acceptance
- Regulatory Issues

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